

VI. CLAIMS

What is claimed is:

1. A method of identifying a set of oligonucleotides that will hybridize with a target nucleic acid comprising, a) determining the dG of oligo-target binding for each potential oligonucleotide that can bind to the target at a determined temperature, b) selecting the oligonucleotides that have a dG of ≤ -30 kcal/mol forming an oligo-target set of oligonucleotides, c) determining the dG at the determined temperature for the intramolecular interactions for the oligonucleotides in the oligo-target set and the dG at the determined temperature for the oligo-oligo intermolecular interactions for the oligonucleotides in the oligo target set, and d) selecting those oligonucleotides from the oligo-target set that have a dG for intermolecular interactions of ≥ -8 kcal/mol and have an intramolecular dG of ≥ -1 kcal/mol, forming a target set of oligonucleotides.
2. The method of claim 1 further comprising the step of determining the length of the each oligonucleotide within the set prior to step a).
3. The method of claim 2, wherein the oligonucleotide length is 20 bases.
4. The method of claim 2, wherein the oligonucleotide length is 21 bases.
5. The method of claim 2, wherein the oligonucleotide length is 30 bases.
6. The method of claim 1, wherein the target temperature is between 25 degrees C to 45 degrees C.
7. The method of claim 1, wherein the target temperature is between 28 degrees C to 42 degrees C.
8. The method of claim 1, wherein the target temperature is between 32 degrees C to 38 degrees C.
9. The method of claim 1, wherein the target temperature is 37 degrees Celsius.
10. The method of claim 1, wherein the target temperature is 25 degrees Celsius.
11. The method of claim 10, wherein step b) comprises selecting the oligonucleotides that have a dG of ≤ -35 kcal/mol forming an oligo-target set of oligonucleotides.
12. The method of claim 1, wherein the target nucleic acid is a consensus sequence of a set of target nucleic acids.
13. The method of claim 12, wherein prior to the step a), the method further comprises the step of determining the consensus sequence of the target nucleic acid.

14. The method of claim 12, wherein the method further comprises the step of adjusting the oligo-target set generated in step a) such that only 99% of the oligonucleotides in the oligo-target set meet the dG requirements.
15. The method of claim 12, wherein the method further comprises the step of adjusting the oligo-target set generated in step a) such that only 95% of the oligonucleotides in the oligo-target set meet the dG requirements.
16. The method of claim 12, wherein the method further comprises the step of adjusting the oligo-target set generated in step a) such that only 90% of the oligonucleotides in the oligo-target set meet the dG requirements.
17. The method of claim 12, wherein the method further comprises the step of adjusting the oligo-target set generated in step a) such that only 80% of the oligonucleotides in the oligo-target set meet the dG requirements.
18. The method of claim 12, wherein the target nucleic acid is a viral nucleic acid.
19. The method of claim 18, wherein the target nucleic acid is RNA.
20. The method of claim 18, wherein the target nucleic acid is DNA.
21. The method of claim 18, wherein the viral nucleic acid is derived from Adenoviridae, Arenaviridae, Astroviridae, Baculoviridae, Barnaviridae, Betaherpesvirinae, Birnaviridae, Bromoviridae, Bunyaviridae, Caliciviridae, Chordopoxvirinae, Circoviridae, Comoviridae, Coronaviridae, Cystoviridae, Corticoviridae, Entomopoxvirinae, Filoviridae, Flaviviridae, Fuselloviridae, Geminiviridae, Hepadnaviridae, Herpesviridae, Gammaherpesvirinae, Inoviridae, Iridoviridae, Leviviridae, Lipothrixviridae, Microviridae, Myoviridae, Nodaviridae, Orthomyxoviridae, Papovaviridae, Paramyxoviridae, Paramyxovirinae, Partitiviridae, Parvoviridae, Phycodnaviridae, Picornaviridae, Plasmaviridae, Pneumovirinae, Podoviridae, Polydnaviridae, Potyviridae, Poxviridae, Reoviridae, Retroviridae, Rhabdoviridae, Sequiviridae, Siphoviridae, Tectiviridae, Tetraviridae, Togaviridae, Tombusviridae, Totiviridae, or variant or strain thereof.
22. The method of claim 18, wherein the viral nucleic acid is derived from Mastadenovirus, Human adenovirus 2, Aviadenovirus, African swine fever virus, arenavirus, Lymphocytic choriomeningitis virus, Ippy virus, Lassa virus, Arterivirus, Human astrovirus 1, Nucleopolyhedrovirus, Autographa californica nucleopolyhedrovirus, Granulovirus, Plodia interpunctella granulovirus, Badnavirus, Commelina yellow mottle virus, Rice tungro bacilliform, Barnavirus, Mushroom bacilliform virus, Aquabirnavirus, Infectious

pancreatic necrosis virus, Avibirnavirus, Infectious bursal disease virus,
 Entomobirnavirus, Drosophila X virus, Alfamovirus, Alfalfa mosaic virus, Ilarvirus,
 Ilarvirus Subgroups 1-10, Tobacco streak virus, Bromovirus, Brome mosaic virus,
 Cucumovirus, Cucumber mosaic virus, Bhanja virus Group, Kaisodi virus, Mapputta
 5 virus, Okola virus, Resistencia virus, Upolu virus, Yogue virus, Bunyavirus, Anopheles
 A virus, Anopheles B virus, Bakau virus, Bunyamwera virus, Bwamba virus, C virus,
 California encephalitis virus, Capim virus, Gamboa virus, Guama virus, Koongol virus,
 Minatitlan virus, Nyando virus, Olifantsvlei virus, Patois virus, Simbu virus, Tete virus,
 Turlock virus, Hantavirus, Hantaan virus, Nairovirus, Crimean-Congo hemorrhagic fever
 10 virus, Dera Ghazi Khan virus, Hughes virus, Nairobi sheep disease virus, Qalyub virus,
 Sakhalin virus, Thiafora virus, Crimean-congo hemorrhagic fever virus, Phlebovirus,
 Sandfly fever virus, Bujaru complex, Candiru complex, Chilibre complex, Frijoles
 complex, Punta Toro complex, Rift Valley fever complex, Salehabad complex, Sandfly
 fever Sicilian virus, Uukuniemi virus, Uukuniemi virus, Tospovirus, Tomato spotted wilt
 15 virus, Calicivirus, Vesicular exanthema of swine virus, Capillovirus, Apple stem
 grooving virus, Carlavirus, Carnation latent virus, Caulimovirus, Cauliflower mosaic
 virus, Circovirus, Chicken anemia virus, Closterovirus, Beet yellows virus, Comovirus,
 Cowpea mosaic virus, Fabavirus, Broad bean wilt virus 1, Nepovirus, Tobacco ringspot
 virus, Coronavirus, Avian infectious bronchitis virus, Bovine coronavirus, Canine
 20 coronavirus, Feline infectious peritonitis virus, Human coronavirus 299E, Human
 coronavirus OC43, Murine hepatitis virus, Porcine epidemic diarrhea virus, Porcine
 hemagglutinating encephalomyelitis virus, Porcine transmissible gastroenteritis virus, Rat
 coronavirus, Turkey coronavirus, Rabbit coronavirus, Torovirus, Berne virus, Breda
 virus, Corticovirus, Alteromonas phage PM2, Pseudomonas Phage phi6, Deltavirus,
 25 Hepatitis delta virus, Dianthovirus Carnation ringspot virus, Red clover necrotic mosaic
 virus, Sweet clover necrotic mosaic virus, Enamovirus, Pea enation mosaic virus,
 Filovirus, Marburg virus, Ebola virus Zaire, Flavivirus, Yellow fever virus, Tick-borne
 encephalitis virus, Rio Bravo Group, Japanese encephalitis, Tyuleniy Group, Ntaya
 Group, Uganda S Group, Dengue Group, Modoc Group, Pestivirus, Bovine diarrhea
 30 virus, Hepatitis C virus, Furovirus, Soil-borne wheat mosaic virus, Beet necrotic yellow
 vein virus, Fusellovirus, Sulfolobus virus 1, Subgroup I, II, and III geminivirus, Maize
 streak virus, Beet curly top virus, Bean golden mosaic virus, Orthohepadnavirus,
 Hepatitis B virus, Avihepadnavirus, Alphaherpesvirinae, Simplexvirus, Human

herpesvirus 1, Varicellovirus, Human herpesvirus 3, Cytomegalovirus, Human
 herpesvirus 5, Muromegalovirus, Mouse cytomegalovirus 1, Roseolovirus, Human
 herpesvirus 6, Lymphocryptovirus, Human herpesvirus 4, Rhadinovirus, Ateline
 herpesvirus 2, Hordeivirus, Barley stripe mosaic virus, Hypoviridae, Hypovirus,
 5 Cryphonectria hypovirus 1-EP713, Idaeovirus, Raspberry bushy dwarf virus, Inovirus,
 Coliphage fd, Plectrovirus, Acholeplasma phage L51, Iridovirus, Chilo iridescent virus,
 Chloriridovirus, Mosquito iridescent virus, Ranavirus, Frog virus 3, Lymphocystivirus,
 Lymphocystis disease virus flounder isolate, Goldfish virus 1, Levivirus, Enterobacteria
 phage MS2, Allolevirus, Enterobacteria phage Qbeta, Lipothrixvirus, Thermoproteus
 10 virus 1, Luteovirus, Barley yellow dwarf virus, Machlomovirus, Maize chlorotic mottle
 virus, Marafivirus, Maize rayado fino virus, Microvirus, Coliphage phiX174,
 Spiromicrovirus, Spiroplasma phage 4, Bdellomicrovirus, Bdellovibrio phage MAC 1,
 Chlamydiamicrovirus, Chlamydia phage 1, T4-like phages, coliphage T4, Necrovirus,
 Tobacco necrosis virus, Nodavirus, Nodamura virus, Influenzavirus A, B and C, Thogoto
 15 virus, Polyomavirus, Murine polyomavirus, Papillomavirus, Rabbit (Shope)
 Papillomavirus, Paramyxovirus, Human parainfluenza virus 1, Morbillivirus, Measles
 virus, Rubulavirus, Mumps virus, Pneumovirus, Human respiratory syncytial virus,
 Partitivirus, Gaeumannomyces graminis virus 019/6-A, Chrysovirus, Penicillium
 chrysogenum virus, Alphacryptovirus, White clover cryptic viruses 1 and 2,
 20 Betacryptovirus, Parvovirinae, Parvovirus, Minute mice virus, Erythrovirus, B19 virus,
 Dependovirus, Adeno-associated virus 1, Densovirinae, Densovirus, Junonia coenia
 densovirus, Iteravirus, Bombyx mori virus, Contravirus, Aedes aegypti densovirus,
 Phycodnavirus, 1-Paramecium bursaria Chlorella NC64A virus group, Paramecium
 bursaria chlorella virus 1, 2-Paramecium bursaria Chlorella Pbi virus, 3-Hydra viridis
 25 Chlorella virus, Enterovirus, Human poliovirus 1, Rhinovirus Human rhinovirus 1A,
 Hepatovirus, Human hepatitis A virus, Cardiovirus, Encephalomyocarditis virus,
 Aphthovirus, Foot-and-mouth disease virus, Plasmavirus, Acholeplasma phage L2,
 Podovirus, Coliphage T7, Ichnovirus, Campoletis sonorensis virus, Bracovirus, Cotesia
 melanoscela virus, Potexvirus, Potato virus X, Potyvirus, Potato virus Y, Rymovirus,
 30 Ryegrass mosaic virus, Bymovirus, Barley yellow mosaic virus, Orthopoxvirus, Vaccinia
 virus, Parapoxvirus, Orf virus, Avipoxvirus, Fowlpox virus, Capripoxvirus, Sheep pox
 virus, Leporipoxvirus, Myxoma virus, Suipoxvirus, Swinepox virus, Molluscipoxvirus,
 Molluscum contagiosum virus, Yatapoxvirus, Yaba monkey tumor virus,

Entomopoxviruses A, B, and C, *Melolontha melolontha* entomopoxvirus, *Amsacta moorei* entomopoxvirus, *Chironomus luridus* entomopoxvirus, Orthoreovirus, Mammalian orthoreoviruses, reovirus 3, Avian orthoreoviruses, Orbivirus, African horse sickness viruses 1, Bluetongue viruses 1, Changuinola virus, Corriparta virus, Epizootic hemarrhagic disease virus 1, Equine encephalosis virus, Eubenangee virus group, Lebombo virus, Orungo virus, Palyam virus, Umatilla virus, Wallal virus, Warrego virus, Kemerovo virus, Rotavirus, Groups A-F rotaviruses, Simian rotavirus SA11, Coltivirus, Colorado tick fever virus, Aquareovirus, Groups A-E aquareoviruses, Golden shiner virus, Cypovirus, Cypovirus types 1-12, *Bombyx mori* cypovirus 1, Fijivirus, Fijivirus groups 1-3, Fiji disease virus, Fijivirus groups 2-3, Phytoreovirus, Wound tumor virus, Oryzavirus, Rice ragged stunt, Mammalian type B retroviruses, Mouse mammary tumor virus, Mammalian type C retroviruses, Murine Leukemia Virus, Reptilian type C oncovirus, Viper retrovirus, Reticuloendotheliosis virus, Avian type C retroviruses, Avian leukosis virus, Type D Retroviruses, Mason-Pfizer monkey virus, BLV-HTLV retroviruses, Bovine leukemia virus, Lentivirus, Bovine lentivirus, Bovine immunodeficiency virus, Equine lentivirus, Equine infectious anemia virus, Feline lentivirus, Feline immunodeficiency virus, Canine immunodeficiency virus, Ovine/caprine lentivirus, Caprine arthritis encephalitis virus, Visna/maedi virus, Primate lentivirus group, Human immunodeficiency virus 1, Human immunodeficiency virus 2, Human immunodeficiency virus 3, Simian immunodeficiency virus, Spumavirus, Human spuma virus, Vesiculovirus, Vesicular stomatitis Indiana virus, Lyssavirus, Rabies virus, Ephemerovirus, Bovine ephemeral fever virus, Cytorhabdovirus, Lettuce necrotic yellows virus, Nucleorhabdovirus, Potato yellow dwarf virus, Rhizidiavirus, Rhizidiomyces virus, Sequivirus, Parsnip yellow fleck virus, Waikavirus, Rice tungro spherical virus, Lambda-like phages, Coliphage lambda, Sobemovirus, Southern bean mosaic virus, Tectivirus, Enterobacteria phage PRD1, Tenuivirus, Rice stripe virus, Nudaurelia capensis beta-like viruses, Nudaurelia beta virus, Nudaurelia capensis omega-like viruses, Nudaurelia omega virus, Tobamovirus, Tobacco mosaic virus (vulgare strain; ssp. NC82 strain), Tobravirus, Tobacco rattle virus, Alphavirus, Sindbis virus, Rubivirus, Rubella virus, Tombusvirus, Tomato bushy stunt virus, Carmovirus, Carnation mottle virus, Turnip crinkle virus, Totivirus, *Saccharomyces cerevisiae* virus, Giardiavirus, *Giardia lamblia* virus, Leishmanivirus, *Leishmania brasiliensis* virus 1-1,

Trichovirus, Apple chlorotic leaf spot virus, Tymovirus, Turnip yellow mosaic virus, Umbravirus, Carrot mottle virus, or variant or strain thereof.

23. The method of claim 12, wherein the viral nucleic acid is derived from Human immunodeficiency virus 1, Human immunodeficiency virus 2, or Human immunodeficiency virus 3.

24. The method of claim 12, wherein the target nucleic acid is a bacterial nucleic acid.

25. The method of claim 24, wherein the bacterial nucleic acid is derived from Abiotrophia, Achromobacter, Acidaminococcus, Acidovorax, Acinetobacter, Actinobacillus, Actinobaculum, Actinomadura, Actinomyces, Aerococcus, Aeromonas, Afipia, Agrobacterium, Alcaligenes, Alloiococcus, Alteromonas, Amycolata, Amycolatopsis, Anaerobospirillum, Anaerorhabdus, Arachnia, Arcanobacterium, Arcobacter, Arthrobacter, Atopobium, Aureobacterium, Bacteroides, Balneatrix, Bartonella, Bergeyella, Bifidobacterium, Bilophila Branhamella, Borrelia, Bordetella, Brachyspira, Brevibacillus, Brevibacterium, Brevundimonas, Brucella, Burkholderia, Buttiauxella, Butyrivibrio, Calymmatobacterium, Campylobacter, Capnocytophaga, Cardiobacterium, Catonella, Cedecea, Cellulomonas, Centipeda, Chlamydia, Chlamydophila, Chromobacterium, Chyseobacterium, Chryseomonas, Citrobacter, Clostridium, Collinsella, Comamonas, Corynebacterium, Coxiella, Cryptobacterium, Delftia, Dermabacter, Dermatophilus, Desulfomonas, Desulfovibrio, Dialister, Dichelobacter, Dolosicoccus, Dolosigranulum, Edwardsiella, Eggerthella, Ehrlichia, Eikenella, Empedobacter, Enterobacter, Enterococcus, Erwinia, Erysipelothrix, Escherichia, Eubacterium, Ewingella, Exiguobacterium, Facklamia, Filifactor, Flavimonas, Flavobacterium, Francisella, Fusobacterium, Gardnerella, Gemella, Globicatella, Gordona, Haemophilus, Hafnia, Helicobacter, Helococcus, Holdemania Ignavigranum, Johnsonella, Kingella, Klebsiella, Kocuria, Koserella, Kurthia, Kytococcus, Lactobacillus, Lactococcus, Lautropia, Leclercia, Legionella, Leminorella, Leptospira, Leptotrichia, Leuconostoc, Listeria, Listonella, Megasphaera, Methylobacterium, Microbacterium, Micrococcus, Mitsuokella, Mobiluncus, Moellerella, Moraxella, Morganella, Mycobacterium, Mycoplasma, Myroides, Neisseria, Nocardia, Nocardiosis, Ochrobactrum, Oeskovia, Oligella, Orientia, Paenibacillus, Pantoea, Parachlamydia, Pasteurella, Pediococcus, Peptococcus, Peptostreptococcus, Photobacterium, Photorhabdus, Plesiomonas, Porphyrimonas, Prevotella, Propionibacterium, Proteus, Providencia, Pseudomonas, Pseudonocardia, Pseudoramibacter, Psychrobacter,

Rahnella, Ralstonia, Rhodococcus, Rickettsia, Rochalimaea, Roseomonas, Rothia, Ruminococcus, Salmonella, Selenomonas, Serpulina, Serratia, Shewenella, Shigella, Simkania, Slackia, Sphingobacterium, Sphingomonas, Spirillum, Staphylococcus, Stenotrophomonas, Stomatococcus, Streptobacillus, Streptococcus, Streptomyces, Succinivibrio, Sutterella, Suttonella, Tatumella, Tissierella, Trabulsiella, Treponema, Tropheryma, Tsakamurella, Turicella, Ureaplasma, Vagococcus, Veillonella, Vibrio, Weeksella, Wolinella, Xanthomonas, Xenorhabdus, Yersinia, and Yokenella. Other examples of bacterium include Mycobacterium tuberculosis, M. bovis, M. typhimurium, M. bovis strain BCG, BCG substrains, M. avium, M. intracellulare, M. africanum, M. kansasii, M. marinum, M. ulcerans, M. avium subspecies paratuberculosis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus equi, Streptococcus pyogenes, Streptococcus agalactiae, Listeria monocytogenes, Listeria ivanovii, Bacillus anthracis, B. subtilis, Nocardia asteroides, and other Nocardia species, Streptococcus viridans group, Peptococcus species, Peptostreptococcus species, Actinomyces israelii and other Actinomyces species, and Propionibacterium acnes, Clostridium tetani, Clostridium botulinum, other Clostridium species, Pseudomonas aeruginosa, other Pseudomonas species, Campylobacter species, Vibrio cholerae, Ehrlichia species, Actinobacillus pleuropneumoniae, Pasteurella haemolytica, Pasteurella multocida, other Pasteurella species, Legionella pneumophila, other Legionella species, Salmonella typhi, other Salmonella species, Shigella species, Brucella abortus, other Brucella species, Chlamydia trachomatis, Chlamydia psittaci, Coxiella burnetii, Escherichia coli, Neisseria meningitidis, Neisseria gonorrhea, Haemophilus influenzae, Haemophilus ducreyi, other Hemophilus species, Yersinia pestis, Yersinia enterocolitica, other Yersinia species, Escherichia coli, E. hirae and other Escherichia species, as well as other Enterobacteria, Brucella abortus and other Brucella species, Burkholderia cepacia, Burkholderia pseudomallei, Francisella tularensis, Bacteroides fragilis, Fudobacterium nucleatum, Provetella species, and Cowdria ruminantium, or any strain or variant thereof.

26. The method of claim 24, wherein the target nucleic acid is RNA.

27. The method of claim 24, wherein the target nucleic acid is DNA.

28. The method of claim 12, wherein the target nucleic acid is a fungal nucleic acid.

29. The method of claim 28, wherein the fungal nucleic acid is derived from Candida albicans, Cryptococcus neoformans, Histoplasma capsulatum, Aspergillus fumigatus, Coccidioides immitis, Paracoccidioides brasiliensis, Blastomyces dermatitidis,

Pneumocystis carinii, Penicillium marneffii, and Alternaria alternate, or strain or variant thereof.

30. The method of claim 28, wherein the target nucleic acid is RNA.

31. The method of claim 28, wherein the target nucleic acid is DNA.

5 32. The method of claim 12, wherein the target nucleic acid is a parasite nucleic acid.

33. The method of claim 32, wherein the parasite nucleic acid is derived from Toxoplasma gondii, Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, other Plasmodium species, Trypanosoma brucei, Trypanosoma cruzi, Leishmania major, other Leishmania species, Schistosoma mansoni, other Schistosoma species, and Entamoeba histolytica, or any strain or variant thereof.

10

34. A composition comprising a primer, wherein the primer comprises the sequence of set forth in position 1042-1065 of SEQ ID NO:1.